MRID No. 427741-10

DATA EVALUATION RECORD

1. **CHEMICAL:** Dicamba.
Shaughnessey No. 029801.

2. **TEST MATERIAL:** Dicamba technical; CAS No. 1918-00-9; Batch No. 52204112; 89.5% active ingredient; a white solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Skeletonema costatum*.

4. **CITATION:** Hoberg, J.R. 1993. Dicamba Technical - Toxicity to the Marine Diatom, *Skeletonema costatum*. SLI Report No. 93-3-4699. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Sandoz Agro, Inc., Des Plaines, IL. EPA MRID No. 427741-10.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
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KBN Engineering and
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6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of technical dicamba, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* were 0.011, 0.033, and 0.493 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. Test Species: The diatom used in the test, *Skeletonema costatum*, came from laboratory stock cultures originally obtained from Bigelow Laboratory, West Boothbay Harbor, ME. Stock cultures were maintained in sterile Artificially Enriched Seawater (AES) medium under test conditions. Transfers were made to fresh medium approximately twice a week. The culture used as the inoculum for the test was transferred to fresh medium eight days before test initiation.

B. Test System: Test vessels were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The vessels were conditioned by rinsing with appropriate test solutions and 50 ml of the test or control solution were placed into each flask. The test medium was the same as that used for culturing with the pH adjusted to 8.1. The salinity was determined to be approximately 30 g/l. Test vessels were randomly placed and maintained on an orbital shaker (shaking rate of 60 rpm) under 16 hours of illumination per day (3.2-4.8 klux at the surface of the medium) in an environmental chamber. The temperature in the chamber was maintained at 20 $\pm$1°C.

C. Dosage: Five-day growth and reproduction test. Based on the results of a range-finding test, six nominal concentrations of 0.0097, 0.032, 0.11, 0.36, 1.2, and 4.0 mg active ingredient (ai)/l were selected for the definitive test. The maximum application rate for dicamba was reported to be 4 lb ai/acre, which is equivalent to 2.9 mg ai/l if applied to a 15-cm water column.

A 40 mg ai/l primary stock solution was prepared by dissolving 0.0200 g (as ai) of test material in AES medium to the final volume of 500 ml. Appropriate volumes of the primary stock solution were diluted to the final volume of 500 ml in AES medium to prepare the treatment solutions. A medium control was also prepared.

D. Test Design: The test consisted of 3 replicate flasks per treatment level and control. An inoculum of *S. costatum* cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask within 25 minutes of solution addition. The inoculum volume was 0.79 ml per flask. At each 24-hour interval, cell health was assessed and counts were conducted on each

replicate vessel using a hemacytometer and compound microscope.

The conductivity and pH were measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer in a flask of water in the environmental chamber. The shaking rate of the orbit shaker and light intensity were recorded daily.

At test initiation and termination, samples were removed from each treatment and control solution for analysis by high performance liquid chromatography. A set of three quality control solutions were prepared at test initiation and termination to monitor the precision and quality control during analysis. Terminal treatment samples were taken from solutions which had been centrifuged (2,000 rpm) for 10 minutes.

E. **Statistics:** The EC_{10}, EC_{50}, and EC_{90} values and their 95% confidence intervals (C.I.) for each 24-hour test period were determined by linear regression of response (percent reduction of cell density as compared with the control) vs. mean measured concentration. Various mathematical manipulations (e.g., logarithm and probit transformations) were used on the concentration and response data to obtain the linear regression with the highest coefficient of determination (R^2). The 95% confidence intervals were determined using the method of inverse prediction.

The no-observed-effect concentration (NOEC) was determined to be the highest concentration that caused no significant reduction of cell density in comparison to the control. Williams' test ($p \leq 0.05$) was used to determine significant effects after first checking the data for normality using Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test.

12. **REPORTED RESULTS:** Mean measured concentrations averaged 103% of nominal (Table 3, attached). The mean measured concentrations were 0.011, 0.033, 0.11, 0.35, 1.2, and 4.1 mg ai/l. Recoveries of the 0- and 120-hour quality control samples averaged 100% of nominal.

Cell densities determined at each observation time are presented in Table 4 (attached). At test termination, mean cell densities in the treatment and control solutions ranged from 38 $x10^4$ to 111 $x10^4$ cells/ml. Cells in the four highest concentration solutions appeared to be fragmented,

bloated, and had thin walls. Cell density was significantly reduced at the five highest concentration levels. Cells at the lowest concentration level appeared normal. The 120-hour NOEC was determined to be 0.011 mg ai/l. The 120-hour EC_{50} was determined to be 0.58 mg ai/l (95% C.I.= 0.090-4.1 mg ai/l).

During the test, conductivity ranged from 41,000 to 43,000 µmhos/cm. The pH was 8.0-8.1 in all treatment and control solutions at test initiation and 8.4-8.6 at test termination.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the study author.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that maintenance of records on the test substance (characterization and verification) is the responsibility of the sponsor. Additionally, routine water analyses were conducted at an independent laboratory that did not collect data in accordance with GLP procedures. A Quality Assurance statement was included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines with the exception that the dilution factor (3.3) was greater than recommended (2.0). Additionally, the light intensity (3.2-4.8 klux) was occasionally lower or higher than recommended (4.0 klux).

B. **Statistical Analysis:** Using EPA's Toxanal program and the mean measured concentration data, the reviewer obtained a more conservative estimate of the EC_{50} using the moving average angle method. The 120-hour EC_{50} and 95% C.I. were 0.49 and 0.29-0.90 mg ai/l, respectively. The reviewer used analysis of variance and Dunnett's test ($p \leq 0.05$) to determine the lowest-observed-effect concentration (LOEC) and NOEC in comparison to the control data. The results were the same as the author's (see attached printouts).

C. **Discussion/Results:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of technical dicamba, the 5-day NOEC,

LOEC, and EC_{50} for *S. costatum* were 0.011, 0.033, and 0.493 mg ai/l, respectively.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 7-27-93.

Table 3. Concentrations of Dicamba measured in the exposure solutions during the 120-hour toxicity test with *Skeletonema costatum*.

	Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L)[a]					
		0-Hour	% Nominal	120-Hour	% Nominal	Mean	% Nominal
	4.0	4.2	110	4.0	100	4.1	100
	1.2	1.2	100	1.2	100	1.2	100
	0.36	0.36	100	0.34	95	0.35	98
	0.11	0.11	100	0.11	97	0.11	100
	0.032	0.033	100	0.032	100	0.033	100
	0.0097	0.011	100	0.010	110	0.011	110
	Control	<0.0049	NA[b]	<0.0049	NA	NA	NA
QC#1[c]	4.00	4.15	104	4.23	106		
QC#2	0.100	0.100	100	0.0965	96.5		
QC#3	0.0100	0.00967	96.7	0.00947	94.7		

[a] Calculated values are based on actual corrected analytical results and not on rounded values (two significant figures) presented in this table.
[b] NA = Not Applicable
[c] QC = Quality Control sample

Table 4. **Cell density (x 10^4 cells/mL) of *Skeletonema costatum* determined after 24-, 48-, 72-, 96- and 120-hours of exposure to Dicamba Technical.**

Mean Measured Concentration (mg A.I./L)		OBSERVATION INTERVAL (HOURS) 24	48	72	96	120
4.1	A	1	11	22	24	32
	B	2	11	23	25	42
	C	1	9	22	22	39
	Mean(SD)[a]	1(<1)[bcd]	10(2)[bcd]	22(1)[bcd]	24(2)[bcd]	38(5)[bcde]
1.2	A	2	10	23	58	55
	B	1	11	29	50	59
	C	3	8	26	45	45
	Mean(SD)[a]	2(1)[bcd]	9(2)[bcd]	26(3)[bcd]	51(6)[bcd]	53(7)[bcde]
0.35	A	2	20	38	57	56
	B	3	16	37	55	53
	C	1	19	35	56	65
	Mean(SD)[a]	2(1)[cd]	18(2)[bcd]	37(1)[bcd]	56(1)[bcd]	58(7)[bcde]
0.11	A	3	24	40	65	61
	B	4	24	40	64	69
	C	4	20	43	67	56
	Mean(SD)[a]	3(1)	23(2)	41(1)	65(2)	62(7)[bcde]
0.033	A	4	24	43	79	84
	B	2	22	40	66	83
	C	3	18	40	82	82
	Mean(SD)[a]	3(1)	21(3)	41(2)	76(8)	83(1)[e]
0.011	A	3	20	45	67	106
	B	3	26	41	85	116
	C	2	23	41	91	108
	Mean(SD)[a]	3(1)	23(3)	42(2)	81(13)	110(5)
Control	A	4	28	49	96	119
	B	4	24	50	76	109
	C	3	22	47	73	107
	Mean(SD)[a]	4(1)	24(3)	49(2)	82(12)	111(7)

[a] Mean and standard deviation (SD) are calculated from original raw data, not from the rounded values presented in this table.
[b] Cell fragments were observed.
[c] Bloated cells were observed.
[d] Thin cell walls were observed.
[e] Statistically reduced ($p \leq 0.05$) as compared to the control based on Williams' Test.

skeletonema cell density
File: skl Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	14867.619	2477.937	75.197
Within (Error)	14	461.333	32.952	
Total	20	15328.952		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

skeletonema cell density
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	111.667	111.667		
2	0.011	110.000	110.000	0.356	
3	0.033	83.000	83.000	6.116	*
4	0.11	62.000	62.000	10.597	*
5	0.35	58.000	58.000	11.450	*
6	1.2	53.000	53.000	12.517	*
7	4.1	37.667	37.667	15.788	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

NOEL= 0.011 mg ai/l
LOEL= 0.033 mg ai/l

skeletonema cell density
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	0.011	3	11.858	10.6	1.667
3	0.033	3	11.858	10.6	28.667
4	0.11	3	11.858	10.6	49.667
5	0.35	3	11.858	10.6	53.667
6	1.2	3	11.858	10.6	58.667
7	4.1	3	11.858	10.6	74.000

MOSSLER DICAMBA SKELETONEMA COSTATUM 7-27-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
4.1	100	66	66	0
1.2	100	52	52	0
.35	100	48	48	0
.11	100	44	44	0
.033	100	25	25	0
.011	100	1	1	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .6480728

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.108261	.492818	.2901788	.9026393

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.4694624	6.116274	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .658356
95 PERCENT CONFIDENCE LIMITS = .2072681 AND 1.109444

LC50 = .6249786
95 PERCENT CONFIDENCE LIMITS = .1597561 AND 11.56934

LC10 = 7.358983E-03
95 PERCENT CONFIDENCE LIMITS = 2.231808E-06 AND 4.426515E-02

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